

## PATENT COOPERATION TREATY



## PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY  
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference <b>NICHI-3</b>	<b>FOR FURTHER ACTION</b> See Form PCT/IPEA/416	
International application No. <b>PCT/JP2004/004214</b>	International filing date ( <i>day/month/year</i> ) <b>25 March 2004 (25.03.2004)</b>	Priority date ( <i>day/month/year</i> ) <b>28 March 2003 (28.03.2003)</b>
International Patent Classification (IPC) or national classification and IPC <b>C12N 15/00, A01H 5/00, C12N 1/19, 1/21, 9/88, C12P 17/02</b>		
Applicant <b>NIHON UNIVERSITY</b>		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

3. This report is also accompanied by ANNEXES, comprising:

a.  (*sent to the applicant and to the International Bureau*) a total of \_\_\_\_\_ sheets, as follows:

sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).

sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

b.  (*sent to the International Bureau only*) a total of (indicate type and number of electronic carrier(s))  
Disc 1, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

<input checked="" type="checkbox"/>	Box No. I	Basis of the report
<input type="checkbox"/>	Box No. II	Priority
<input type="checkbox"/>	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
<input type="checkbox"/>	Box No. IV	Lack of unity of invention
<input checked="" type="checkbox"/>	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
<input type="checkbox"/>	Box No. VI	Certain documents cited
<input type="checkbox"/>	Box No. VII	Certain defects in the international application
<input type="checkbox"/>	Box No. VIII	Certain observations on the international application

Date of submission of the demand <b>15 April 2004 (15.04.2004)</b>	Date of completion of this report <b>17 March 2005 (17.03.2005)</b>
Name and mailing address of the IPEA/JP	Authorized officer
Facsimile No.	Telephone No.

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/JP2004/004214

## Box No. I Basis of the report

1. With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

- This report is based on translations from the original language into the following language \_\_\_\_\_, which is language of a translation furnished for the purpose of:
- international search (under Rules 12.3 and 23.1(b))
  - publication of the international application (under Rule 12.4)
  - international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the elements of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

- The international application as originally filed/furnished

- the description:

pages \_\_\_\_\_ received by this Authority on \_\_\_\_\_  
 pages\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_  
 pages\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_

- the claims:

pages \_\_\_\_\_, as originally filed/furnished  
 pages\* \_\_\_\_\_, as amended (together with any statement) under Article 19  
 pages\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_  
 pages\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_

- the drawings:

pages \_\_\_\_\_, as originally filed/furnished  
 pages\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_  
 pages\* \_\_\_\_\_ received by this Authority on \_\_\_\_\_

- a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3.  The amendments have resulted in the cancellation of:

- the description, pages \_\_\_\_\_
- the claims, Nos. \_\_\_\_\_
- the drawings, sheets/figs \_\_\_\_\_
- the sequence listing (*specify*): \_\_\_\_\_
- any table(s) related to sequence listing (*specify*): \_\_\_\_\_

4.  This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- the description, pages \_\_\_\_\_
- the claims, Nos. \_\_\_\_\_
- the drawings, sheets/figs \_\_\_\_\_
- the sequence listing (*specify*): \_\_\_\_\_
- any table(s) related to sequence listing (*specify*): \_\_\_\_\_

\* If item 4 applies, some or all of those sheets may be marked "superseded."

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/JP2004/004214

**Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement****Novelty (N)**

Claims 1-50 YES

Claims \_\_\_\_\_ NO

**Inventive step (IS)**

Claims 24-48 YES

Claims 1-23, 49-50 NO

**Industrial applicability (IA)**

Claims 1-50 YES

Claims \_\_\_\_\_ NO

**2. Citations and explanations (Rule 70.7)****Document 1**

AKASHI T, ET AL., New Scheme of the Biosynthesis of Formononetin Involving 2,7,4'-Trihydroxyisoflavanone but Not Daidzein as the Methyl Acceptor. Biosci Biotechnol Biochem (2000) Vol. 64, No. 10, p. 2276-2279

Based on the description in document 1 cited in the international search report, the inventions of claims 1-23, 49, and 50 lack an inventive step.

Document 1 describes the biosynthesis of formononetin by the action of a cultured liver cell extract on 2,7,4'-trihydroxyisoflavanone, and it states that formononetin is produced by the action of the liver cell extract in the presence of a yeast expressing isoflavanone synthase, flavanone, and a methyl group donor. Document 1 also suggests that based on the mechanism of this reaction, an enzyme catalyzing methylation of the 4 position of 2,7,4'-trihydroxyisoflavanone and a 2,7-dihydroxy-4'-methoxyisoflavanone dehydratase are present in the cultured liver cell culture material.

Generally speaking, when an organism possessing enzymatic activity with a specific function is known, it is common practice to purify the enzyme having that activity by conventional means such as a suitable combination of column chromatography, etc., determine the N-terminal amino acid sequence of that enzyme and the amino acid sequence of a portion different from the N-terminus, and clone a gene encoding a polypeptide having that enzymatic activity from a genomic DNA library or cDNA library prepared from the original organism using a DNA primer prepared from those amino acid sequences. Therefore, this examination finds that persons skilled in the art can easily predict the existence of 2,7-dihydroxy-4'-methoxyisoflavanone dehydratase, purify a protein having 2,7-dihydroxy-4'-methoxyisoflavanone dehydratase activity, clone the gene that encodes 2,7-dihydroxy-4'-methoxyisoflavanone dehydratase using the above widely known means, insert that cloned DNA into a suitable transformant, and using that transformant to produce 2,7-dihydroxy-4'-methoxyisoflavanone dehydratase. Moreover, this examination finds that the same presents no particular technical difficulty.

## Supplemental Box Relating to Sequence Listing

## Continuation of Box No. 1, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis that of:

## a. type of material

- a sequence listing  
 table(s) related to the sequence listing

## b. format of material

- in written format  
 in computer readable form

## c. time of filing/furnishing

- contained in the international application as filed  
 filed together with the international application in computer readable form  
 furnished subsequently to this Authority for the purpose of search and/or examination  
 received by this Authority as an amendment\* on \_\_\_\_\_

2.  In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

\* If item 4 in Box No. I applies, the listing and /or table(s) related thereto, which form part of the basis of the report, may be marked "superseded".

## Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of Box V:

Furthermore, this examination finds that obtaining a genomic DNA and cDNA library from that organism, screening for a transformant having the desired activity from among suitable host cells into which that DNA has been introduced, and analyzing the gene inserted into the obtained transformant having the desired activity are likewise commonly performed activities. Therefore, persons skilled in the art can easily prepare a genomic or cDNA library from liver based on the description in document 1, transform suitable host cells, select transformants having 2,7-dihydroxy-4'-methoxyisoflavanone dehydratase activity, and obtain the gene that encodes 2,7-dihydroxy-4'-methoxyisoflavanone dehydratase by analyzing the inserted genes.

The inventions of claims 24-48 involve an inventive step with respect to the documents cited in the international search report.

None of the documents cited in the international search report describes or suggests that 2,7,4'-trihydroxyisoflavanone dehydratase is present in soybeans, and more specifically they do not describe nor suggest 2,7,4'-trihydroxyisoflavanone dehydratase having the amino acid sequence identified as SEQ ID NO: 3 and the gene encoding 2,7,4'-trihydroxyisoflavanone dehydratase having the nucleotide sequence identified as SEQ ID NO: 4.